IV SUMMARY

The focus of this thesis was on the assembly of the Pex1p-Pex6p-complex from S. cerevisiae, which is crucial for the peroxisomal matrix protein import and thus peroxisome biogenesis. Following statements can be made based on the experimental data obtained in this thesis:

(1) The peroxisomal AAA-type ATPases Pex1p and Pex6p from S. cerevisiae can successfully be expressed in E. coli Tuner (DE3) and purified in high quality and sufficient amounts for functional as well as structural analyses.

(2) The recombinant Pex1p-Pex6p-complex behaves like the in vivo complex on a size exclusion chromatography column and displays an ATPase activity with a \( k_m \) of 0.17 mM and a \( V_{\text{max}} \) of 0.35 nmol min\(^{-1}\) µg\(^{-1}\). Both aspects indicate the functionality of the recombinant complex. Moreover, the observed ATPase activity of the recombinant Pex1p-Pex6p-complex can be inhibited by NEM.

(3) Size exclusion chromatography studies of the individual proteins and the Pex1p-Pex6p-complex suggest that three Pex6p monomers and one Pex1p trimer assemble into the heterohexameric AAA-complex.

(4) ATP turned out to be not only important for complex assembly and maintenance but is also required for the stability of the individual proteins. These data were obtained by purification of the AAA-proteins in absence of any nucleotides and by depletion of ATP after purification of the recombinant Pex1p-Pex6p-complex in presence of ATP. Moreover, NEM most likely leads to dissociation of the complex by inhibition of ATP-binding to the second AAA-domain of Pex1p.

(5) Analysis of the AAA-complex in presence of different nucleotides revealed that the hexameric Pex1p-Pex6p-complex can also exist in an ADP-bound state, but is less stable than in presence of ATP. This observation indicates conformational changes within the Pex1p-Pex6p-complex during its ATPase cycle.

(6) Interaction studies of truncated Pex1p with full-length Pex6p suggest that the second AAA-domain of Pex1p is required for the formation of a stable Pex1p-Pex6p-complex.

(7) Pex1p and Pex6p can successfully be chemically cross-linked by DSS and BS\(^3\). This serves as basis for the identification of direct contact sites between those two proteins using mass spectrometry.